



## Description of *Poecilia (Acanthophaecelus) obscura* n. sp., (Teleostei: Poeciliidae), a new guppy species from western Trinidad, with remarks on *P. wingei* and the status of the “Ender’s guppy”

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### Abstract

*Poecilia obscura*, new species, is described from the Oropuche system, Trinidad. A mitochondrial DNA-sequence based molecular phylogenetic analysis revealed the status of the new species as a separate taxon. It is most closely related to the Common guppy, *P. reticulata* and to the recently described species, *P. wingei*. It can also be distinguished by morphometrics and gonopodial characteristics from these two species, although the ranges for all values overlap. A definition of the new species on morphology criteria alone is thus impossible. Therefore, *P. obscura* forms a cryptic species complex with the two other species. *P. wingei* is now unequivocally defined by the molecular phylogeny as a valid species. The three guppy species are included in the subgenus *Acanthophaecelus* Eigenmann (1907), which is considered as generically different from all other taxa of the Poeciliinae sensu Parenti (1981).

**Key words:** molecular phylogeny, sexual dimorphism, freshwater fish, artificial introduction, hybridization, cryptic species complex

### Introduction

Fish that are commonly known as guppies are one of the most widely used model systems and laboratory animals. They also became a favorite ornamental fish for aquarium hobbyists due to the possibility to create fancy colorful morphs with exquisite fin forms. Their popularity in the pet trade and use in mosquito control has resulted in guppies being released worldwide outside their natural range in the northeastern part of South America and adjacent Caribbean Islands.

For a long time the guppy was considered monophyletic and hence a monotypic taxon, *Poecilia reticulata* (Peters, 1859). Allozyme and DNA sequence based studies revealed a marked genetic differentiation among guppy populations (Alexander and Breden, 2004; Carvalho *et al.*, 1991; Russell and Magurran, 2006; Shaw *et al.*, 1991). In particular, this phenomenon was most obvious for fish from the Caroni vs. the Oropuche drainages of Northern Trinidad and for guppies from certain habitats around the city of Cumaná (also known as Ender’s guppy) vs. other populations from Venezuela. This genetic differentiation was interpreted as being the consequences of independent cases of incipient speciation in *P. reticulata* (Alexander and Breden, 2004; Russell and Magurran, 2006). The observed phenomenon was strongly argued as being an instance of “evolution in action” where population differentiation has not led to the formation of species (Magurran, 1998).

Poeser *et al.* (2005) described exclusively on the basis of morphological and behavioral characters a second species of guppy from the Paria peninsula (Venezuela) as *Poecilia wingei* and hypothesized that the Cumaná guppies used in the above mentioned genetic studies might be local populations of *P. wingei* or even

hybrids with *P. reticulata*, but did not validate their taxonomic classification in the light of the “genetic differentiation without speciation” hypothesis. We describe here the guppies from the Oropuche system of Trinidad as a third species of *Poecilia*, subgenus *Acanthophaecelus*, and show by molecular phylogenetic analysis that all three guppy species are old species, which arose by parallel evolution rather than being emerging new species.

Peters first described the guppy as *Poecilia reticulata* in 1859 on the basis of material collected by the pharmacist J. Gollmer from the Guayre River in or near Caracas (Peters, 1859). Independently Günther described *Girardinus guppii* as a valid younger synonym from material sent to him by the Trinidadian naturalist R. J. Lechmere Guppy (Günther, 1866). Regan (1913) without further explanation classified the species to the genus *Lebistes*. He considered the type description of the species, *Lebistes poecilioides* (De Filippi, 1861) (material from Barbados, collected by Reverend Ermenegildo Arnaboldi) as another synonym of *P. reticulata*. Rosen & Bailey (1963) reclassified *L. reticulatus* to the genus *Poecilia*. Since Poeser & Isbrücker (2002) have shown that *Lebistes poecilioides* does not represent a guppy, the generic name *Acanthophaecelus* is now available. The later taxon was established by Eigenmann (1907) as *Acanthophaecelus reticulatus* with *P. reticulata* as type species. Poeser *et al.* (2005) proposed on the basis of synapomorphies between *P. reticulata* and *P. wingei* to revalidate *Acanthophaecelus* as subgenus. We follow this provisional subclassification but suggest that a revision of the current genus *Poecilia* that remained after the reestablishment of *Limia* (Hamilton, 2001; Rodriguez, 1997) and *Micropoecilia* (Meyer, 1993) is necessary in the future.

The subgenus *Acanthophaecelus*, which so far included *P. reticulata* and *P. wingei*, has a wide range in northeastern South America and some Caribbean islands. *P. reticulata*, is known from a variety of habitats. The natural distribution of the common guppy is not exactly known, but we follow Rosen & Bailey (1963) listing the Netherlands Antilles, Venezuelan islands, Trinidad, Windward (Barbados) and Leeward (St Thomas and Antigua) islands, Western Venezuela (and adjacent parts of Columbia in the Rio Orinoco drainage) to British Guiana (= Guyana). Magurran added Surinam and probably Tobago to the natural range and pointed out that it is unclear whether localities in Barbados, Cuba, and Brazil have been naturally colonized by *P. reticulata* or are the result of human introductions (Magurran, 2005, and pers. comm.). *P. wingei* has been reported from brackish waters in the Campoma-Carúpano-region, Paría Peninsula, Venezuela. Our investigations show that *P. wingei* also occurs in the Cumaná region, Venezuela.

Here, we describe the guppies of North-East Trinidad, which so far were regarded as a population of *P. reticulata* as a separate species of the subgenus *Acanthophaecelus*.

## Materials and methods

Material of *P. reticulata*, *P. wingei* and the new species from Trinidad stemmed from a field trip to Trinidad in February 2008, and from the fish collections of Dr. David Reznick, UC Riverside, California, USA, Dr. Joanne Cable, Cardiff University, Great Britain, and Dr. Christine Dreyer, Max Planck Institute, Tübingen, Germany. Material of *P. wingei* from the type locality stemmed from the fish collection of Dr. Fred Poeser, University of Amsterdam, Netherlands. The maculatus-zebrinus strain of *P. reticulata* was obtained from Prof. Dr. J.-H. Schröder, formerly at GSF, München, Germany. Ornamental guppies were purchased from the aquarium trade from import material. All material used in this study is listed in table 1 and geographic origins are depicted in figure 1.

Type material of the new species is deposited at the Museum für Tierkunde, Dresden, and Senckenberg Museum, Frankfurt, Germany.

Measurements were made by vernier calipers, reading to 0.1 mm. Measurements and counts follow standard practice (Miller, 1948). The number of specimens for all counts is greater or equal to 15. The length of the distal tip of gonopodium ray 4a and 3 is measured on a horizontal line from the distal tip of ray 4p to the distal tip of the gonopodium hook. The depth of gonopodium is measured on a vertical line from ray 3 to ray

**TABLE 1.** Origins and sources of fishes.

| Specimen                  | Geographical origin  | Remarks   |
|---------------------------|--|---|
| <i>P. obscura</i> OR      | Oropuche river (N 10° 43.052'; W 61° 8.871')                           | Road to Cumaca caves, locus typicus   |
| <i>P. obscura</i> O2      | Oropuche, headwaters   | Laboratory strain "Oropuche 2", established by D. Reznick                           |
| <i>P. obscura</i> RS      | Río Seco River   | Below Río Seco waterfall, at junction with Salybia River                            |
| <i>P. obscura</i> Q6      | Quare, below Hollis Reservoir  | Laboratory strain "Quare 6", established by D. Reznick                              |
| <i>P. obscura</i> Ma      | Matura River tributary (N 10° 40.523'; W 61° 4.407')                   | 1 km north of road from Sangre Grande to Matura,                                    |
| <i>P. obscura</i> LE      | L'Ebranche tributary (N 10° 30.528'; W 61° 4.204')                     | 3 km south of Upper Manzanilla, road to Plum Milan                                  |
| <i>P. wingei</i> Ca       | Campoma, division between Laguna Buena Vista and Laguna Campoma        | 3 km North of Cariaco, locus typicus for <i>P. wingei</i> ; supplied by F.N. Poeser |
| <i>P. wingei</i> LP       | Laguna de los Patos (N 10° 25.043'; W 64° 12.065')                     | West Cumaná   |
| <i>P. wingei</i> CC       | Central Cumaná (N 10° 26.5'; W 64° 11.5')                              | Laboratory strain "Endler Cumaná", EnCCFR (see Alexander & Breden 2004)             |
| <i>P. cf. "wingei"</i> EC | El Cordon (N 10° 28.242'; W 63° 33.116')                               | Below El Cordon waterfall, Represa El Cordon, Cariaco                               |
| <i>P. reticulata</i> RG   | Río Guayre (N 10° 20.396'; W 66° 36.369')                              | Tributary to Río Guayre, near La Mocha  |
| <i>P. reticulata</i> CS   | Caroni swamp (N 10° 32.013'; W 61° 26.797')                            | West Trinidad   |
| <i>P. reticulata</i> ZMB  | Río Guayre near Caracas Museum für Naturkunde, Berlin, coll. #ZMB 6299 | Original material collected by J. Gollmer   |
| "Girardinus guppii"       | Trinidad, Museum für Naturkunde, Berlin, coll. #ZMB 6081               | Paratype, collected by R. J. Lechmere Guppy   |
| <i>P. reticulata</i> RY   | Rio Yaguaracual (N 10° 18.463'; W 64° 21.338')                         | Venezuela   |
| <i>P. reticulata</i> YR   | Upper Yarra River  | Headwaters of Yarra River, Northern Trinidad  |
| <i>P. reticulata</i> MR   | Upper Marianne River   | Headwaters of Marianne River, Northern Trinidad                                     |
| <i>P. reticulata</i> Hi   | Hillsborough   | Tobago  |
| <i>P. reticulata</i> Sp   | Speyside   | North-East Tobago   |
| <i>P. reticulata</i> PL   | Pitch Lake   | South-West Trinidad   |
| <i>P. reticulata</i> Ka   | Kaw  | French Guyana   |
| <i>P. reticulata</i> RV   | Rio Vichada  | Columbia  |
| <i>P. reticulata</i> Sa   | Salimaos   | Brazil  |
| <i>P. reticulata</i> SA   | St. Anns   | Northeastern suburb of Port of Spain, West Trinidad                                 |
| <i>P. reticulata</i> RZ   | Rio Zuata, near Requena  | Northern tributary to Orinoco, Venezuela  |
| <i>P. reticulata</i> RT   | Rio Tefe   | Brazil  |

continued next page

**TABLE 1.** (continued)

| Specimen                    | Geographical origin                                | Remarks   |
|-----------------------------|--|---|
| <i>P. reticulata</i> IM     | Isla de Margarita                                  | Venezuela   |
| <i>P. reticulata</i> ma-ze  | unknown  | Laboratory strain “maculatus-zebrinus”, established by O. Winge |
| <i>P. reticulata</i> CB     | Claxton Bay  | South-West Trinidad   |
| <i>Micropoecilia picta</i>  | Laguna de los Patos (N 10° 25.043'; W 64° 12.065') | West Cumaná   |
| Ornamental guppy YSDF       | Aquarium trade                                     | Variety: Yellow snakeskin, delta fantail                        |
| Ornamental guppy JBDS       | Aquarium trade                                     | Variety: Japan blue, double sword                               |
| Ornamental guppy redBRDF    | Aquarium trade                                     | Variety: half black, red delta fantail                          |
| Ornamental guppy blue BBDF  | Aquarium trade                                     | Variety: blue, blue delta fantail                               |
| Ornamental guppy green GGDF | Aquarium trade                                     | Variety: green, green delta fantail                             |

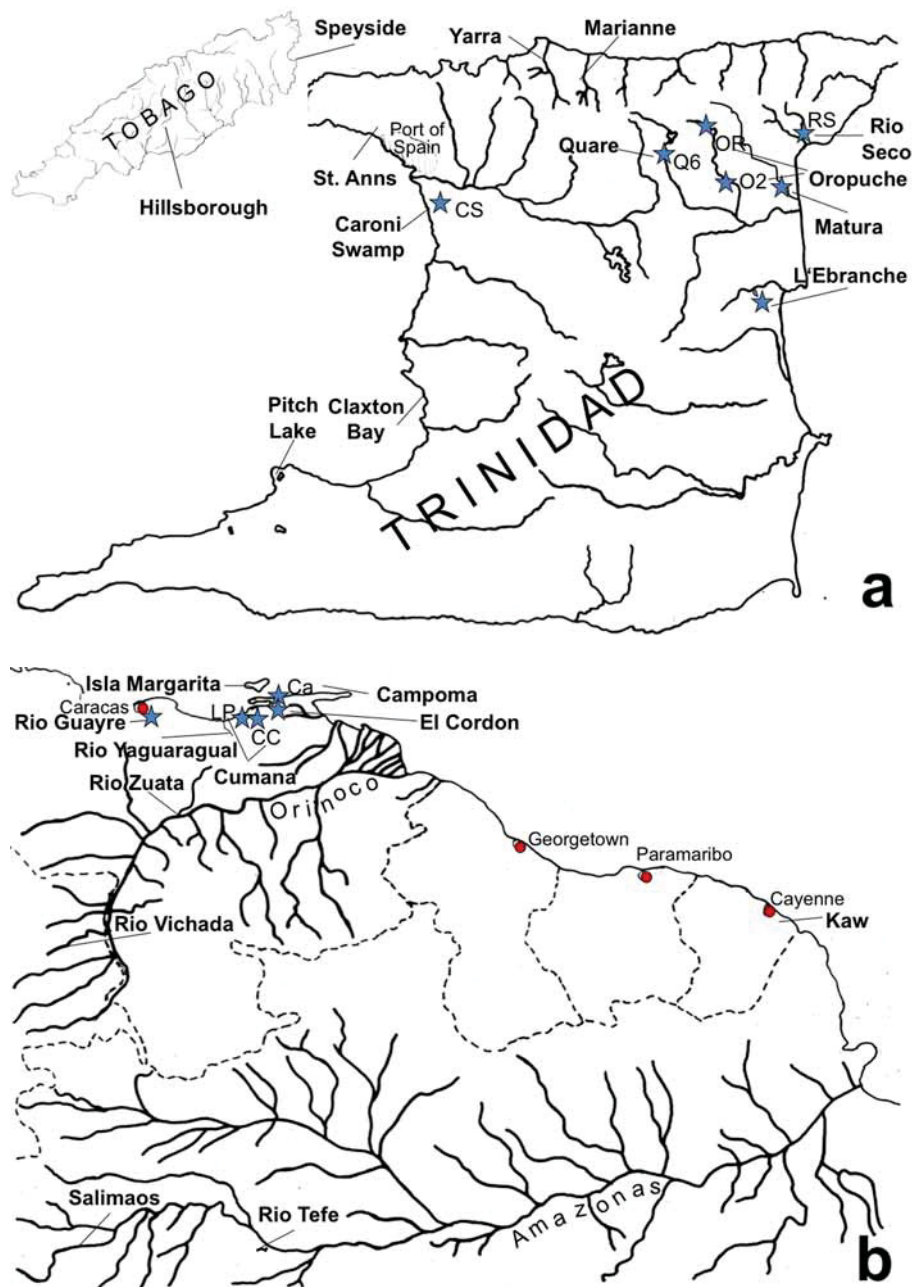
5p exactly where the serrae on 4p starts to nestle against ray 4a. The total gill-raker count of the first gill arch includes all gill rakers in the angle of the gill arch. The last two rays of the dorsal fin are counted as a single ray. Vertebral counts include the hypural plate as one vertebra. The nomenclature of the sensory canal system of the head follows the standard of Gosline (Gosline, 1949) and elements of the gonopodial system follow Rosen & Gordon, Rosen & Kallman and Rosen & Bailey (Rosen and Bailey, 1963; Rosen and Gordon, 1953; Rosen and Kallman, 1959).

Genomic DNA was isolated from pooled organs of individual fish as described by Scharf *et al.* (Scharf *et al.*, 1995) or from dorsal fin clips according to Altschmied *et al.* (Altschmied *et al.*, 1997). Sequences from the mitochondrial control (D-loop) region and the *cytochrome b* gene were amplified by PCR. For the control region the primer pairs L15513 (5'CTGGGAGACCCNGAAAACCTT3')/ H16498 (5'CCTGAAGTAGGAACCAGATG3') and L15995 (5'AACTCTCACCCCTAGCTCCCAAAG3')/ H693 (5'GGCGGATACTTGCATGT3') and for *cytochrome b* the primer pair L14724 (5'CGAAGCTTGATATGAAAAACCATCGTTG3')/ H15982 (5'CCTAGCTTTGGGAGYTAGG3') were used. The PCR was done under the following conditions: denaturation 95 °C for 30 sec, annealing 52 °C (for D-loop primers L15995/H16498), 54 °C (for D-loop primers L 15513/H16100) or 47 °C (for *cytochrome b*) for 30 sec, extension 72 °C for 45 sec. 34 cycles were run from less than 100 ng genomic DNA. In each case a single PCR product was obtained and sequenced directly by GATC Biotech. The resulting data set consists of approximately 2200 bases for each species. For comparison to other populations, laboratory strains and museum specimens shorter sequences (approx. 800 bases) were generated. Nucleotide sequences were analyzed using the program BioEdit Sequence Alignment Editor (copyright <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). Multiple sequence alignments were generated using the same program. Phylogenetic analyses were done with MEGA 3.1 (Tamura *et al.*, 2007). Heuristic and branch-and-bound tree searches were performed using both parsimony and distance (minimum evolution). Neighbor-joining (NJ) trees were also constructed. Robustness of the trees was tested by bootstrap analyses using 20000 replicas All DNA sequences generated in this study are deposited in GenBank under accession numbers GQ 855708 - 855741.

### ***Subgenus Acanthophaelus* Eigenmann, 1907**

The subgenus *Acanthophaelus* is characterized by the following derived characters (S1) very long and massive recurved hook present on tip of gonopodium ray 5, hook size longer than depth of the 4p-serrae; (S2) numerous (> 10) long and pointed comb-like spines of gonopodium ray 3; (S3) high degree of black, white,

green, blue, yellow and red color polymorphism on body sides and dorsal and tail fins of adult males, females generally lacking prominent markings on body sides except for rare individuals with single irregular black stripes or spots at the caudal fin base or body side.



**FIGURE 1.** Origins of guppies used in this study from Trinidad and Tobago (a), and mainland South America (b). Stars indicate exactly known collection sites, all other designations are for rivers or areas from which the fish were collected.

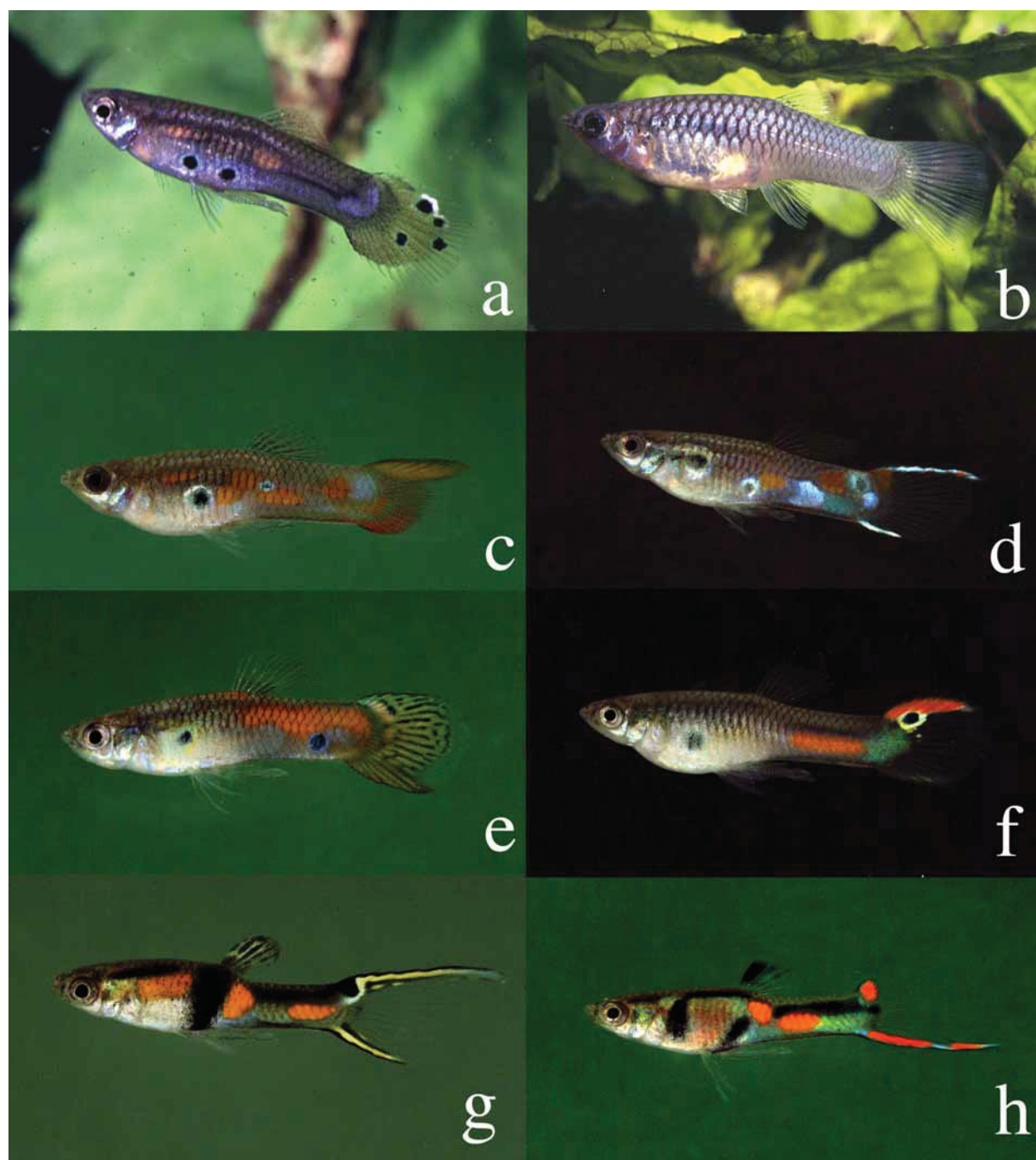
***Poecilia (Acanthophaeus) obscura*, new species**  
(Figs. 2a–c, 3, 4, 6–8, table 2)

Holotype. Male (MTD F31503), 15.1 mm SL, for other measures see table 2; Oropuche River (N 10° 43.052' W 61° 8.871') below the bridge where the road from Cumaca to Cumaca Caves crosses the river approx 2.5 km past Cumaca, Trinidad; February 13<sup>th</sup> 2008, S. Schories, M. Scharl, P. Fischer leg.



Paratypes. 3 males, 5 females (MTD F31504 – 31511); for measures see table 2; same collection as holotype. 4 males, 2 females (SMF 31068); for measures see table 2; Rio Seco, below Rio Seco waterfall, at junction with Salybia River, Trinidad, February 17<sup>th</sup> 2008, S. Schories, M. Scharl, P. Fischer leg.

**Diagnosis.** *Poecilia obscura* (Fig. 2a–c, 3, table 2) is a small poeciliid species from the subgenus *Acanthophaeus* (SL in males usually not exceeding 20 mm and in females 30 mm), which is distinguished from all other species of the subgenus by the following characters: comb-like spines of gonopodium ray 3 not numerous (11 to 15), basal massive, vs. numerous (14 to 18), basal slender in *P. reticulata* and *P. wingei* (Fig. 3). Being genetically divergent from the other species of the subgenus, the definition of the new species is mainly based on evidence from DNA sequence.



**FIGURE 2.** *P. obscura* male (a) and female (b) from locus typicus, Oropuche River (OR). (c) *P. obscura*, population Rio Seco (RS); (d) *P. reticulata*, population Caroni Swamp (CS); (e) *P. reticulata*, population Rio Yaguaracual (RY); (f) *P. cf* “wingei” from El Cordon waterfall (EC); (g) *P. wingei* from locus typicus, population Campoma (Ca); (h) *P. wingei*, population Laguna de los Patos (LP).

*P. obscura* is also recognized by morphometrics: caudal peduncle short in females (SL/CPD = 4.8 to 6.15), vs. long (SL/CPD = 6.05 to 7.4) in *P. reticulata* and *P. wingei* (SL/CPD = 6.5 to 7.3) (Fig. 4); usually 6 dorsal fin rays, vs. usually 7 in *P. reticulata* and *P. wingei*.

**Description.** Body deep in females, head long and not sharply pointed, 25.5–26.5% of SL. Longitudinal scale series 24 to 25 (rarely 24); predorsal scale series 12 to 13; scale series around caudal peduncle 14. Number of vertebrae 25 to 27. Gill rakers on first arch 11 to 12.

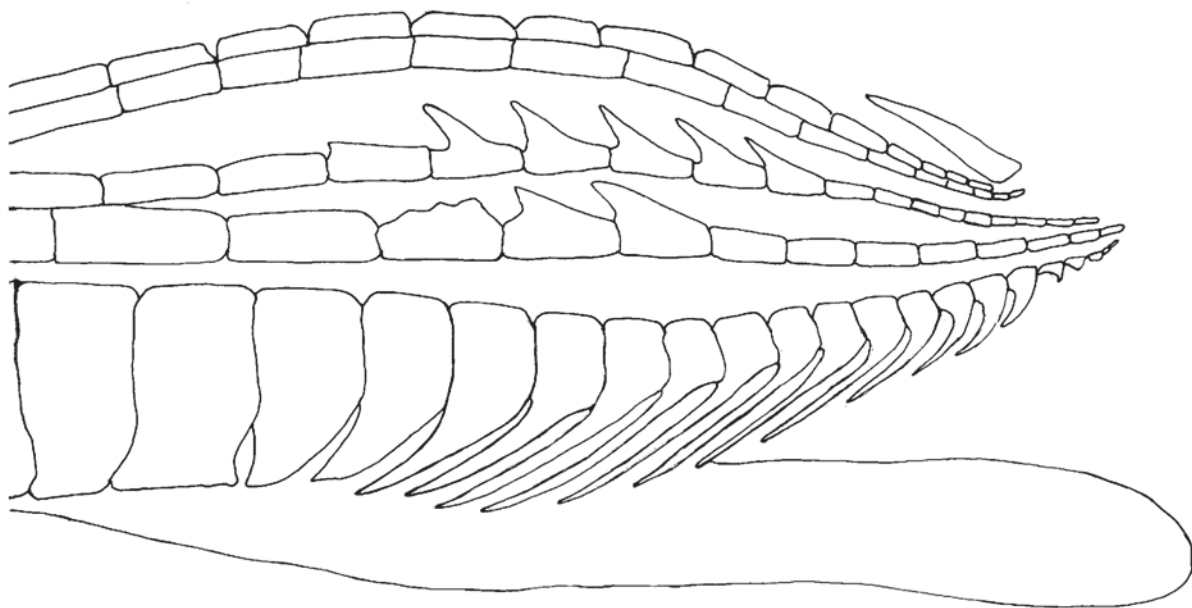
Teeth of upper and lower jaws unicuspid and recurved; those of outer row enlarged and spatula-shaped, numerous and widely spaced, the much smaller posterior teeth have a single sharp spine. Upper pharyngeal bones balloon shaped. Teeth of the medial region somewhat enlarged, each side with a series of 8 rows, teeth small and conical. Lower pharyngeal bone (ceratobranchial 5) heart-shaped and with very numerous pointed and slender unicuspid teeth, 17 to 19 on posterior rows, 5 to 6 on middle rows. Teeth of medial region somewhat enlarged. Arms of pharyngeal very short and split at the ends. Ceratobranchial 4 without teeth, hypobranchial 4 present.

Supraorbital canal system well developed, section 1 to 2a and 2b to 4a, usually represented as grooves, 6b to 7 as pits; preopercular canal with 6 pores; preorbital canal with 4 pores (sometimes represented as groove).

Gonopodium (Fig. 3) short and compact, 2.9 to 3.1 times in SL; ray 3 broadly expanded, curved distally and with widely spaced, 11 to 13 comb-like spines not terminating in a hook; ray 4a curved distally, with a small serra, well developed broad and long segments; ray 4p distally somewhat curved and with massive developed serrae, proximal serrae with 3 to 5 thorns, rays 5a and 5p straight, distal with a large recurved boomerang-shaped bony hook. Rays 6 and 7 long and somewhat thickened distally and curved dorsally.

Gonopodial suspensorium with two well developed gonapophyses, gonapophyses I and II long and slender, each with an uncinus. Ligastyle absent or not well developed. Gonactinost 1 with a small inferior wing-like appendage, gonactinostal complex 2 to 4 in front with a superior small lateral wing, gonactinosts 5 to 9 without bony plates or outgrowths.

Small dorsal fin with 6 to 7 rays (first ray simple, all others branched), origin of dorsal fin posterior to the insertion of anal fin; caudal fin with 22 to 23 rays (10–13 rays branched); anal fin 9 rays (6 rays branched); pectoral fin with 14 rays (9 rays branched); ventral fin with 6 rays (first and last ray simple, all others branched), in females not reaching to the anal fin base and in males reaching widely over the base of the gonopodium.



**FIGURE 3.** Gonopodium of *P. obscura*

Males and females with sex specific coloration (Fig. 2a,–c). Body color of adult females grayish brown to yellow, fins hyaline; body sides of adult males with red, blue, orange and yellow bright pigment spots, some reflecting iridescent, usually with 1 to 3 rounded black spots, sometimes with a series of irregularly thin and short or long brown or light black horizontal lines or with very short brown vertical lines sometimes crossing the horizontal bars; the caudal fin base often shows a lower or upper black spot surrounded by small dark and short dashes and yellow pigment, dorsal or ventral caudal fin rays sometimes pigmented and rarely elongated over the caudal margin of the fin, forming a short “sword”; dorsal fin often whitish, dark or polychromatic colored, sometimes flag-like elongated, all other fins hyaline. Male body coloration extremely polymorphic: in natural habitats no two males being alike. Body coloration and caudal appendix phenotype predominantly heritable characters, male offspring of a single male in laboratory crosses being very similar to their father and to each other.

**TABLE 2.** Measurements of holotype and paratypes of *Poecilia (A.) obscura* **sp. n.**

|       | #           | TL   | SL   | HL  | SNL | BD  | IOW | GL  | ED  | APL | CPL | CPD | PL  | VL  | PDL  |
|-------|-------------|------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| HT(M) | MTD F 31503 | 21.3 | 15.1 | 4.2 | 1.2 | 4.1 | 1.9 | 4.4 | 1.3 |     | 4.9 | 2.9 |     | 3.5 | 9.9  |
| PT(F) | MTD F 31504 | 33.4 | 25.9 | 6.0 | 1.4 | 7.3 | 3.4 |     | 2.1 | 9.6 | 8.9 | 4.4 | 5.6 | 3.3 | 16.6 |
| PT(F) | MTD F 31505 | 35.8 | 28.3 | 6.2 | 1.5 | 7.7 | 3.6 |     | 2.2 | 9.9 | 9.3 | 4.7 | 5.6 | 3.4 | 17.1 |
| PT(F) | MTD F 31506 | 32.6 | 25.5 | 5.7 | 1.4 | 7.2 | 3.3 |     | 2.1 | 8.3 | 8.0 | 4.3 | 5.3 | 3.2 | 16.3 |
| PT(F) | MTD F 31507 | 28.9 | 21.7 | 5.3 | 1.3 | 5.8 | 2.8 |     | 1.8 |     |     | 3.8 |     |     |      |
| PT(M) | MTD F 31508 | 24.4 | 17.8 | 4.6 | 1.3 | 4.8 | 2.3 | 4.6 | 1.5 | 3.7 | 6.4 | 3.3 |     | 3.7 | 10.6 |
| PT(F) | MTD F 31509 | 21.1 | 16.2 | 4.3 | 1.3 | 4.2 | 2.1 |     | 1.4 |     |     | 3.2 |     |     | 10.2 |
| PT(M) | MTD F 31510 | 21.4 | 15.3 | 4.2 | 1.3 | 4.2 | 2.0 | 4.5 | 1.4 |     |     | 2.7 | 4.2 | 3.6 | 9.8  |
| PT(M) | MTD F 31511 | 19.2 | 13.9 | 4.0 | 1.1 | 3.9 | 1.7 | 4.2 | 1.2 |     | 4.6 | 2.7 | 4.0 | 3.4 | 9.6  |
| PT(M) | SMF 31068   | 24.0 | 17.7 | 4.5 | 1.3 | 4.9 | 2.3 | 4.7 | 1.5 | 3.8 | 6.3 | 3.3 | 4.9 | 3.7 | 10.7 |
| PT(F) | SMF 31068   | 32.7 | 25.4 | 6.1 | 1.4 | 7.0 | 3.4 |     | 2.1 | 9.7 | 9.4 | 4.2 | 5.4 | 3.3 | 16.4 |
| PT(F) | SMF 31068   | 33.5 | 26.1 |     |     | 7.2 | 3.5 |     | 2.1 | 9.5 | 9.5 | 4.5 | 5.5 | 3.3 | 16.5 |
| PT(M) | SMF 31068   | 25.6 | 18.2 | 4.7 | 1.3 | 5.0 | 2.4 | 4.9 | 1.6 |     |     | 3.6 |     | 3.9 | 10.9 |
| PT(M) | SMF 31068   | 24.4 | 17.9 | 4.5 | 1.2 |     | 2.3 | 4.7 | 1.5 | 3.8 | 6.2 | 3.5 |     | 3.8 | 10.5 |
| PT(M) | SMF 31068   | 20.4 | 14.7 |     | 1.1 | 4.0 | 1.8 | 4.3 | 1.3 |     |     | 2.8 |     |     | 9.4  |

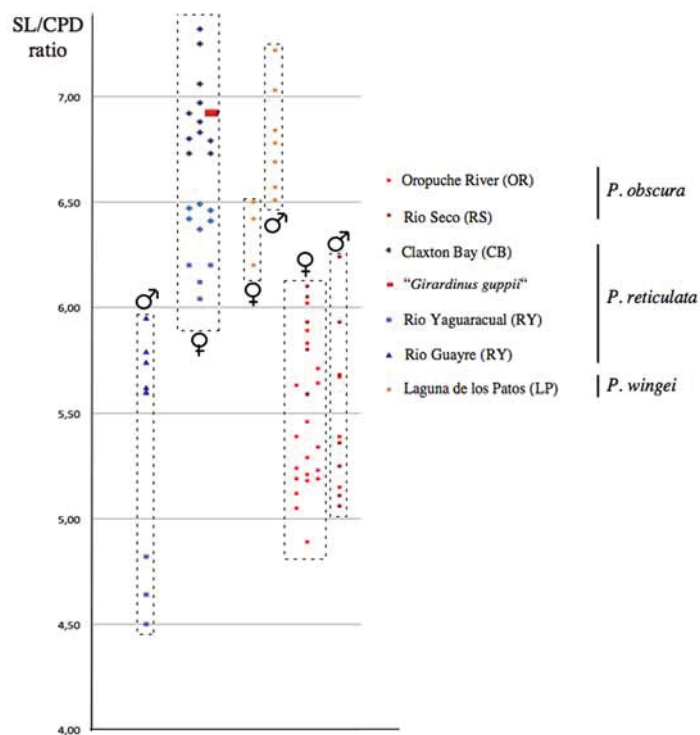
all values are in mm, HT holotype, PT paratype, M male, F female.

**Etymology.** The name of the new species is derived from the latin word obscurus (hidden) because of its status as a cryptic species.

For the common name we propose to call *P. obscura* the Oropuche guppy due to its main area of distribution. For *P. reticulata*, Poeser et al (Poeser *et al.*, 2005) suggested the name Common or Orinoco guppy. For *P. wingei* they proposed the name Campoma guppy; reflecting the narrow range on the Paria peninsula where they found the species. After it is now clear on the basis of our molecular analysis that the species also occurs in the Cumaná region and that it represents Endler’s guppy, it makes sense to return to the priority common name of Cumaná or Endler’s guppy (Alexander and Breden, 2004).

**Habitat notes.** *P. obscura* was collected from fast flowing small rivers and ditches with generally clear waters. The species also occurs in downstream habitats. Thus it inhabits both so-called low and high predation sites described in the guppy literature (see Magurran, 2005). The locus typicus (Fig. 5) is a site in the headwaters of the Oropuche River. The river is here about 5 meters wide and fast flowing with a maximum depth of 1 meter. The riverbed is gravel and sand with some larger rocks in the middle. Submerge plants are absent, but the vegetation on the river banks focally reaches into the water and provides shaded sections throughout most of the day. On the 13.2.2008 (1 p.m., air temperature 25.5°C) the following parameters were recorded: clear water, water temperature 22.5°C, hardness 5–10, conductivity 51 mS, pH 7, nitrate 0, nitrite 0. Accompanying fishes were *Rivulus hartii*, *Astyanax spec.*, *Ancistrus spec.* and small cichlids. The inclusion of cichlids and characins defines the habitat as high predation site.





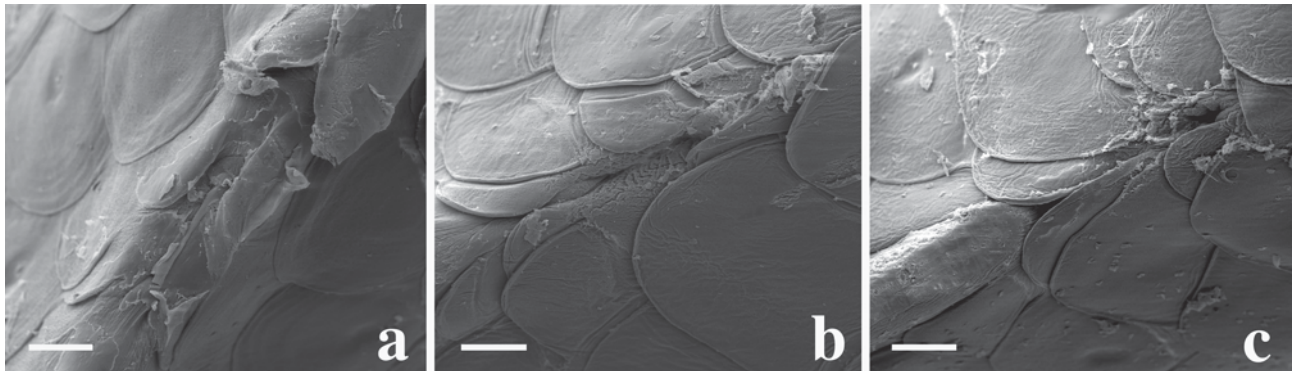
**FIGURE 4.** CPD/SL/CPD ratios of *P. obscura* (symbols, population Rio Seco (RS), population Oropuche (OR)) and *P. reticulata* females (symbols, population Claxton Bay (CB) population Caroni Swamp (CS)).



**FIGURE 5.** Upper Oropuche river, locus typicus for *P. obscura*.

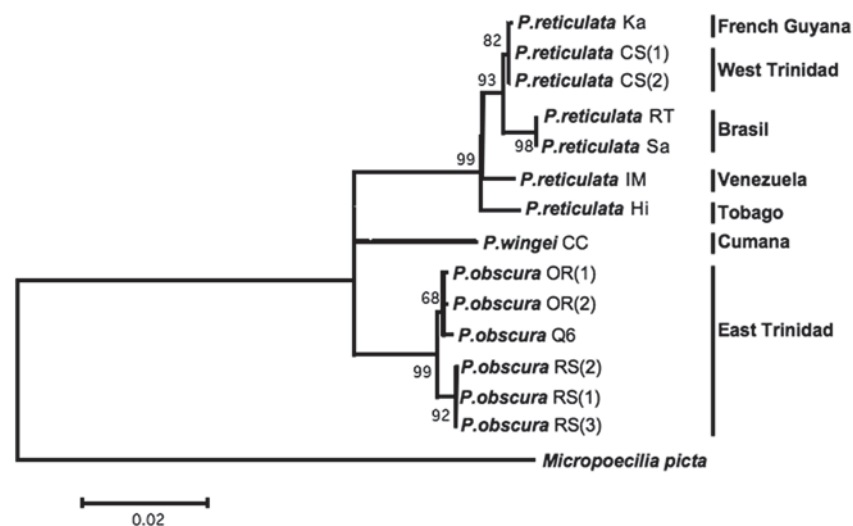
**Comparison, relationships and discussion.** On the basis of synapomorphies, *P. obscura* is unequivocally attached to the subgenus *Acanthophaelus*. By morphological criteria *P. obscura* is most

closely related to *P. reticulata* and *P. wingei*. There are several synapomorphies that unite the three species, namely: large recurved hook present on tip of gonopodium ray 5 and serrae of ray 4p and 4a well developed, long and pointed comb-like spines of gonopodium ray 3 starting very close to the tip. Male pigmentation colorful and extremely polymorphic, more brilliant, metallic and less variable in *P. wingei*. We noted no difference in the morphology of the genital pore of the females from the three species (Fig. 6).



**FIGURE 6.** Scanning electron microscopy images of female genital pores of (a) *P. wingei* (Laguna de los Patos), (b) *P. reticulata* (Caroni Swamp), (c) *P. obscura*, (Upper Oropuche). Bar represents 200  $\mu$ m.

However, *P. obscura* is recognized as a separate species, because it does not share the following characters with *P. reticulata* and *P. wingei*: fewer subdistal comb-like spines of gonopodium ray 3 (11–15, vs. 14–18), higher and shorter caudal peduncle in females, apparent as lower SL/CPD values (Fig. 4). In *P. obscura* SL/CPD values of males are in the same range as females while there is a sexual dimorphism for this value in *P. reticulata*. In this species the SL/CPD ratios of males are lower than for females and in the same range as males of *P. obscura*. Whether also *P. wingei* is polymorphic for this character could not be conclusively determined from the available specimen and requires inspection of the type material in the future.



**FIGURE 7.** Phylogram of *P. obscura*, and *P. reticulata* from different localities and *P. wingei* based on approx. 2200bp of mitochondrial DNA sequences. 50% majority rule consensus tree rooted on *M. picta* as outgroup. Average bootstrap values obtained using different types of analysis (see Materials and Methods) are indicated above the branches. For abbreviations of origins see table 1. Numbers in brackets indicate different individuals from the same locality.

The female of the paratypes of “*Girardinus guppii*” (ZMB 6081) that were sent by Dr. Günther to his colleague at the Museum in Berlin was also measured. These fish are designated as “origin Trinidad”, however, no further information is available on the exact collection place or even river system. The SL/CPD

value of this fish is close to the average for *P. reticulata*. It has to be considered, however, that with respect to morphometrics substantial shape differences have been noted between different populations (Alexander *et al.*, 2006; Hendry *et al.*, 2006). Only when more populations of *P. obscura* can be analyzed in the future it will become clear how robust these characters are and how suitable they will be for determining the taxonomic status of the species.

A molecular phylogenetic analysis was performed using mitochondrial D-loop and cytochrome *b* sequences. The molecular data were analyzed with maximum likelihood, parsimony and neighbor-joining methods which yielded almost identical phylogenetic results. The topology of the resulting trees was always the same.

The phylogram rooted on *Micropoecilia picta* has three well-separated branches (Figs. 7, 8), which are supported by high bootstrap values. One branch is built by the sequences of *P. wingei* from the locus typicus and several collections from the city of Cumana. A second branch consists of the *P. obscura* sequences. The third branch represents *P. reticulata*. Here all other guppy sequences cluster that span the entire mainland range of the species from Columbia to Brazil and the islands of Tobago and Isla Margarita. Importantly the West Trinidadian guppies from the Caroni drainage are also firmly nested in this branch.

We also obtained sequences from a specimen of *P. reticulata* (ZMB 6299) of the collection of Julius Gollmer from which the type material for the first description was taken. This fish was collected and preserved on the 14.5.1856 in the Rio Guayre near the city of Caracas (Venezuela). This sequence clusters with all present-day *P. reticulata* including a fish taken in 2008 from a tributary to the Guayre (RG) near Caracas (Fig 8).

It is a well-known fact that guppies evolve extremely rapidly in response to natural selection (Magurran, 1998; Reznick *et al.*, 1997). This phenomenon could be partially explained by a very high natural mutation rate, which in turn could compromise the significance of the molecular analysis. We therefore included in our dataset five different ornamental guppy strains. These fish independently have been under the most extreme selection for several polygenic traits under enforced short generation times by guppy breeders in Russia, Taiwan, Thailand and Singapore. On the molecular level they were, however, indistinguishable from wild-caught guppies (Fig. 8).

We included also a laboratory strain of *P. reticulata* that is kept as closely inbred line and displays a very homogeneous male pigmentation phenotype. This strain, designated maculatus-zebrinus (ma-ze), was established by Ojvind Winge in the twenties of the last century. Its mitochondrial DNA sequence is nested within all the other *P. reticulata*.

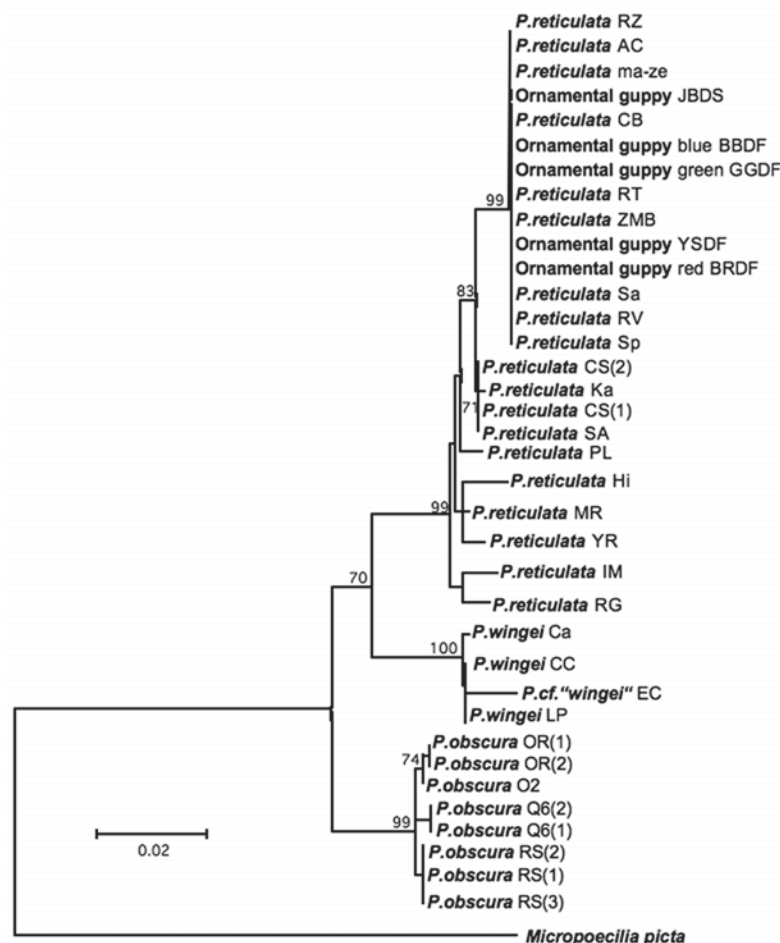
The new species is described mainly on the basis of molecular data. Although the differentiation of the three branches of guppies is unequivocal and indicates separated (mitochondrial) gene pools it may be questioned whether the genetic distance is in the range that usually is found between species that are more classically defined by morphological criteria. To evaluate this, the genetic distances and branch lengths between the three guppy species were compared to the values obtained in studies of other poeciliid fish groups where also mitochondrial control region and the cytochrome *b* gene were used for phylogeny construction. The genetic distance separating the three guppy taxa is 0,029. This is well in the range of values obtained for morphologically very different species in the genus *Xiphophorus* (0.029 between Southern platyfish species, *X. maculatus*, *X. milleri*, and Southern swordtails, e.g. *X. hellerii*, *X. signum* (Meyer and Scharl, 2002); 0,018 between the Southern swordtail species, e.g. *X. hellerii*, *X. signum*, *X. alvarezi* (Meyer and Scharl, 2003); 0,025 between *Priapella olmecae*, *chamulae*, *compressa* and *intermedia* (Scharl *et al.*, 2006); 0,038 between the shortfin mollies of the genus *Poecilia* (Meyer *et al.*, 2004)).

Because the three species of the subgenus *Acanthophaeus* are not readily or reliably distinguishable on a morphological basis but can be separated using non-morphological data, such as from DNA sequence analysis, they can be regarded as another example of a cryptic species complex.

Although the validity of estimating divergence times from DNA sequence variations has been debated (e.g. Howell *et al.*, 2008) it can give at least a reasonable estimate, in particular when calibrations are available from related groups of organisms as is the case for the mitochondrial control region and cytochrome

b genes of actinopterygian fishes (for a recent compilation see BurrIDGE *et al.*, 2008). Using the fastest and slowest rates of all known calibrated molecular clocks in teleosts the separation of the three guppy species should have occurred between 2.5 and 5 million years ago based on the cytochrome b sequences (0.0076 – 0.0036 changes/site/Myr) and between 0.4 and 4.2 million years based on the sequences of the control region (0.044 – 0.004).

*P. obscura* is endemic to the island of Trinidad. A genetic divergence of the guppies from the Oropuche drainage has been noted earlier using mitochondrial DNA sequences or allozyme data, (Alexander and Breden, 2004; Carvalho *et al.*, 1991; Russell and Magurran, 2006); (Alexander *et al.*, 2006). In all phylogenetic trees published so far by these authors the so-called “Oropuche guppies” formed a separate branch. But this was never discussed on basis of recognizing these fish as a separate species. Moreover the differentiation between the fish from the Caroni and the Oropuche drainages was interpreted as the two subgroups being on their way to establish different species, but not having completed the whole process. Our analyses presents a different view, namely of two species that have been separated since a long time. The Caroni drainage, which drains west into the gulf of Paria is separated by a watershed from the Oropuche drainage although the minimum distance between the two river systems during the rainy season can be as close as 70m (Magurran, 2005). The Oropuche and some other rivers with *P. obscura* flow east and drain into the Atlantic. Notably, there is a third drainage, namely those rivers that drain north into the Caribbean Sea. Interestingly these fish, although clearly representing the species *P. reticulata* somehow cluster in the phylogenetic trees with *P. reticulata* from the northward-located island of Tobago (Fig. 8), indicating a possible colonization from Tobago towards Northern Trinidad or vice-versa.



**FIGURE 8.** Phylogramm of *P. obscura* and *P. reticulata* and *P. wingei* from different localities based on approximately 800 bp of cytochrome b DNA sequences. 50% majority rule consensus tree rooted on *M. picta* as outgroup. Average bootstrap values obtained using different types of analysis (see Materials and Methods) are indicated above the branches. For abbreviations of origins see table 1. Numbers in brackets indicate different individuals from the same locality.



The hypothesis that the Caroni (including the Northern rivers) and Oropuche drainages have an independent freshwater fauna from different ancestral lineages is supported by studies on reptiles (Boos, 1984), the cyprinodont fish *Rivulus hartii* (Jowers *et al.*, 2008) and paleogeographic evidence. During the glaciation periods of the Pleistocene sea levels were up to 130 m lower and Trinidad was connected to the mainland (Kenny, 1988). The Orinoco, the main distribution area of *P. reticulata*, at that time reached further northward and was probably connected to the Caroni drainage, discharging to the Gulf of Paria. The Oropuche drainage had most likely confluence with a separate more easterly river system and was colonized from there. Thus *P. obscura* and *P. reticulata* arrived from different regions to Trinidad and did not cross the watershed that still separates them in the Northern mountain range. We hypothesize that *P. wingei* was the most western species but recently was circled by northwest colonizing populations of *P. reticulata*.

Poeser *et al.* (2005) argued that the Eastern Trinidad guppies, now *P. obscura*, originally were the same species as *P. wingei* and have been “despeciated” through hybridization and genetic introgression by the Common (or Orinoco) guppy, *P. reticulata*. Our molecular analysis does not support this interpretation, as there is absolutely no indication of a closer relationship of *P. obscura* with *P. wingei*.

Given the strong variation in male pigmentation the description of *P. wingei* on the basis of coloration was not conclusive. But our molecular data now define the species unequivocally and show that it is a separated taxon from *P. reticulata* and *P. obscura*. Clearly, all fish earlier classified and recognized as Endler’s guppies from localities in and around Cumana are *P. wingei*.

The molecular barcode from mitochondrial sequences will help to define the actual species ranges and hybrid zones. Evidently fish from collecting station 6 of Poeser *et al.* (2005) were designated by these authors as *P. reticulata* on the basis of their male coloration phenotype. We revisited this place (El Cordon, EC). The molecular analysis revealed clearly that these fish have the *P. wingei* mitochondrial sequence (Fig. 8). No further data exist to clarify whether this discrepancy can be explained by interspecific hybridization. We also noted as reported by Poeser *et al.* (2005) that in the river below the El Cordon waterfall several males had an unusual long gonopodium. However, closer inspection revealed that all of these were immature males and that the gonopodium, once fully differentiated is not longer than of any other guppy. Only in the transition period of the anal fin to the intromittent male genitalia a prolongation of the fin happens. This can be seen - although in a less extreme degree - in any other guppy strain. It is unclear whether this is an environmental effect, because such an extreme lengthening of the transforming anal fin was not observed anymore in the subsequent captivity reared offspring.

Male coloration is much more uniform in *P. wingei* from Cumana and is much alike the pigmentation of the fish from Campoma and all earlier known Endler’s guppies (Fig. 2g,h). We suspect that fish (see Fig. 2f) collected by Poeser *et al.* (2005) including those from the El Cordon waterfall, which have a “similar color pattern as the Orinoco variety” (= *P. reticulata*), but have additionally some metallic pigment spots like *P. wingei* from the Locus typicus and the Cumana populations (CC, LP) may be hybrids. Future studies that include nuclear genetic markers will be necessary to clarify this.

All other fish that based on molecular data could be unequivocally assigned to the *P. wingei* clade were of a typical coloration distinct (see also Fig. 2g,h) from *P. obscura* and *P. reticulata*, which we describe here as: longitudinal broad red band extending maximally from the operculum to the caudal fin base, along or above the lateral midline in the trunk and along or below the lateral midline in the peduncle, often interrupted by a vertical black bar originating around the anal fin base and extending to the dorsal fin base or anterior thereof. The red band often dissolved into oval or rarely round red spots, especially in the peduncle. A black stripe above the red band extending maximally to the eyes and the caudal fin base, sometimes missing in the trunk area. Additional dark black coloration of the ventral margin of the peduncle in many males. Caudal fin often with ventral and dorsal sword-like coloration, red, yellow or white, often with a black margin, frequently upper and/or lower rays of caudal fin prolonged to form colored swords or double-swords. Some males with large black shoulder spot with fuzzy margin, similar to the vertical bar. Perfectly round dark black body spots with sharp borders, which are typical for *P. reticulata* and *P. obscura* males, very rare. Large roundish or oval bright metallic blotches of, green, or more rarely yellow or light blue iridescent color following the basic

longitudinal pattern interrupting the red band in the peduncle. White markings rare on the body sides. Rarely small black irregular spots on the belly. Dorsal fin hyaline, yellow and black coloration, sometimes anterior and dorsal margin black. This typical coloration has been documented extensively by Alexander & Breden (2004) for the populations from Central Cumana and we found this also for the Laguna de los Patos (West Cumana) and Campoma populations.

*P. obscura* was most probably not recognized so far as a separate species, because these fish are not different from *P. reticulata* in the most obvious character of guppies, namely male coloration and pigmentation pattern (Fig. 2a, c–e), most of them being linked to sex chromosomes. This is different from *P. wingei*, which because of its more bright pigmentation became suspicious to John Endler and was an issue of debate since then. Nevertheless *P. wingei* males also share many components of their pigmentation patterns with males from the two other species, suggesting that this is an ancestral trait. This is comparable to the situation in the genus *Xiphophorus*, where the (mostly sex chromosome linked) macromelanophore pattern can be found in similar expression phenotype in different species; or in the genus *Poecilia* where the male coloration is almost alike in the sailfin mollies.

As mentioned above the genetic difference between *P. obscura* from East Trinidad and *P. reticulata* from West Trinidad has been realized in numerous studies using allozyme, microsatellites and mitochondrial DNA sequences (Carvalho *et al.*, 1991; Russell and Magurran, 2006; Alexander and Breden, 2004). The absence of obvious morphological criteria and the fact that hybridization between both forms occurs readily in the Turure River as a consequence of the Haskins transplant has led to the conclusion that both forms are rather in an early stage of speciation. Russell and Magurran (2006) noted that laboratory produced hybrids between *P. reticulata* from West Trinidad and *P. obscura* display male behavioral sterility and hybrid breakdown for embryo viability, brood size and sperm counts. This indicates some genetic separation which was interpreted as an initial stage rather than what can be expected from long separated species. The extent to which interspecific hybrids show features of hybrid breakdown can vary, however, and is not especially prominent in other poeciliid species (Schartl, 2008). Moreover, interspecific hybrids can be produced in the laboratory of most poeciliids tested so far. Prezygotic isolation mechanisms are especially efficient to prevent interspecific hybridization of naturally co-occurring species of the same genus, but may not exist in allopatric species. Thus the Turure explant might have created an artificial situation of “sympatry” like enforced breeding of allopatric species in the laboratory. A similar situation exists for *P. wingei*, which was not recognized as a species for a long time although strong genetic differentiation and differences in male display traits, body shape and coloration were clearly documented (Alexander and Breden, 2004). The presence of hybrids in the Cumana area as a result of human activities showing the Y-chromosome dominated phenotype of *P. wingei* but having the mitochondrial genome of *P. reticulata* might have confused the interpretation of the analyses.

**Distribution.** *P. obscura* occurs in freshwaters of the Oropuche River system, North-eastern Trinidad. The species was found (confirmed by DNA sequencing) in the headwaters and upper regions of the northern tributaries to the Oropuche River including the Quare River and La Seiva River. It was also found in the Matura River, the Salybia /Rio Seco Rivers and the L'Ebranche River, which are not tributaries to the Oropuche River but flow independently next north and south into the Atlantic Ocean at the West Coast of Trinidad. The southern and northern boarder of the range of the new species have not been determined exactly, but there are guppies in the Tompire River that flows north of the Matura River and in the Mission River, which flows into the Caribbean Sea (Reznick, pers. comm.). Further west on the northern coastline all the rivers that flow into the Caribbean Sea have no guppies until the Madamas River is reached (Reznick, pers. comm.). This river belongs to the northwestern province, the guppies of which - according to the molecular phylogeny - clearly are *P. reticulata*. No information is available for the situation south of the L'Ebranche.

In 1957 C.P. Haskins released 200 *P. reticulata* from the lower Guanapo River of the Caroni drainage (West Trinidad) into a site of the upper Turure (Magurran, 2005), a northern tributary to the lower Quare River that flows into the lower Oropuche. These fish spread out from there downstream in the Turure (Shaw *et al.*, 1992) (Magurran, 2005). We noted by presence of the *P. reticulata* mitochondrial haplotype (data not shown)

that the invaders have already reached the main river and could threat the newly described species either by displacement or hybridization. Hybrids between *P. reticulata* from the Caroni system (West Trinidad) and *P. obscura* are easily produced in the laboratory (Russell and Magurran, 2006) and a gene flow from the transplantation site into the native population of the Turure was already noted in a microsatellite study (after Russel 2004, cited in Magurran, 2005)). Due to this problem the dispersal of *P. obscura* in the main river of the Oropuche system including its southern tributaries is unclear.

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## Abbreviations

APL = distance anus to pectoral fin; BD = depth of body; CPL = length of caudal peduncle; CPD = depth of caudal peduncle; ED = diameter of eye; F = female; GL = length of gonopodium; HL = length of head; HT = holotype; IOW = interorbital width; LDG = length of gonopodium tip; M = male; MTD = fish collection of the Museum fuer Tierkunde, Dresden, Germany; PDL = predorsal length; PL = length of pectoral fin; PT = paratype; S = synapomorphy, SMF = fish collection of the Forschungsinstitut und Museum Senckenberg, Frankfurt/Main, Germany; SNL = length of snout; SL = standard length; TL = total length; VL = length of ventral fin.